Project Report: Searching for ancestral sequences

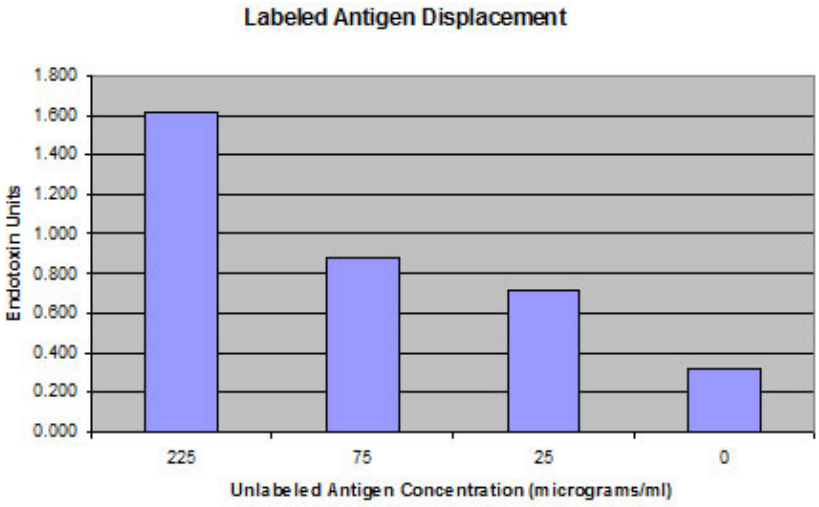
Project Investigators: ***Rebecca Gast , Norman Wainwright***

Project Progress

A major objective of Astrobiology is to search for signs of life in the Solar System and beyond. While remote sensing of biological signatures is a major target for identifying promising sites for future missions, direct analysis of samples for the presence of biomarkers of interest will continue to be an important area for technology development.

This project's main goal is to link the amplification of the Limulus Amebocyte Lysate (LAL) enzyme cascade to biomarkers associated with microbial life, enabling rapid and sensitive detection of life *in situ* . A major sub–goal is to "Use the existing prototype instrument design to collect kinetic spectrophotometric data from competitive labeled antibody assays."

We have demonstrated feasibility of linking lipopolysaccharide (LPS) to an antigen, as the first step toward accomplishing this goal. Fluorescein coupled LPS was captured by anti–fluorescein antibody immobilized on a polystyrene plastic surface. Exposure of the captured label to unlabeled fluorescein resulted in a proportion LAL signal, shown in Figure 1.



Proportional displacement of LPS coupled fluorescein is able to be measured

in Endotoxin Units by LAL after exposure to increasing unlabelled antigen. Assay sensitivity in this example in the microgram range. This relatively low sensitivity is likely due to the low specific activity of the LPS coupled antigen. Future work will improve ratio of LPS label per molecule. We will use a periodate oxidation of the LPS molecule to covalently couple to protein and peptide target sequences. This chemistry will also be attempted with nucleic acid probes.

We have also established a collaboration with Andrew Steele, Carnegie Institution of Washington (CIW) Astrobiology Center . Part of that collaboration was a study of the performance of immunoassays in microgravity (KC−135 flights) that will have direct bearing on our project (Maule, et al., J. Gravitational Physiology, in press 2004). Contrary to our initial concern, antigen/antibody binding was unaffected and in some tests, enhanced in microgravity. We hypothesize that greater liquid mixing may be occurring in microgravity. Collaboration with CIW will link development of our enhanced LAL−coupled immunoassay to CIW's "Lab on a Chip" technology development.

Highlights

- Feasibility of linking LPS to a specific antigen for amplified detection by LAL was demonstrated.
- A collaboration with Andrew Steele at the Carnegie Institution of Washington was established, linking our project to the MASSE “Lab on a Chip” project.

Roadmap Objectives

- ***Objective No. 7.1:*** Biosignatures to be sought in Solar System materials
- ***Objective No. 7.2:*** Biosignatures to be sought in nearby planetary systems

Cross Team Collaborations

Andrew Steele, Carnegie Institution, Washington DC Identified as a collaborator to incorporate this project with MASSE project "Lab on a Chip"